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Molecular mimicry between *Helicobacter pylori* and the host

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Helicobacter pylori lipopolysaccharide (LPS) expresses Lewis x and Lewis y blood group antigens that are identical to those occurring in the human gastric mucosa. During infection, antibodies against LPS, which bind to host Lewis antigens, may be induced. These consequently recognize gastric glycoprotein targets and cause autoimmune inflammation.

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Helicobacter pylori is involved in the pathogenesis of gastritis, peptic ulcer disease, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma[1]. The mechanisms by which infection with this bacterium leads to these various diseases are under intensive investigation. In 1991, it was demonstrated immunohistochemically that *H. pylori* infection may induce serum antibodies that react with the gastric antral mucosa[2]. The reactivity of these antibodies was directed to the mucus layer lying on the mucosa and to the foveolar and glandular epithelial cells. In later studies[3,4], autoreactivity with the corpus mucosa was demonstrated, including binding to parietal cells (Fig. 1). The reactivity with parietal cells is at the level of the canaliculi[3,4], which are parts of the parietal luminal membrane that protrude intracellularly and increase the interface between the parietal cell and the gastric lumen. The canaliculi also contain the gastric H^+K^+-ATPase (i.e. the gastric proton pump responsible for acid production in the stomach), which is well recognized as a target in the autoimmune gastritis associated with pernicious anaemia[5].

As preabsorption of patient sera with *H. pylori* cells abolishes crossreaction with the gastric mucosa[2,3], the existence of molecular mimicry between *H. pylori* and the host mucosa has been postulated. However, until recently, the nature of the putative crossreacting antigen(s) was unknown.

Molecular mimicry of host structures by microorganisms can have pathogenic consequences; infection may induce antibodies and T cells to bacterial cell components that can also recognize self, and immune-mediated damage may follow. This can be illustrated by the development of Guillain–Barré Syndrome (GBS), a neurodegenerative disease that can occur after infection with *Campylobacter jejuni*[6]. Core structures within the lipopolysaccharide (LPS) of certain *C. jejuni* serotypes mimic host neuronal gangliosides. Antibodies to *C. jejuni* LPS may bind to peripheral nerves and thus play a pivotal role in the pathogenesis of GBS. By analogy, *H. pylori*-induced, crossreactive antibodies may contribute to gastric mucosal damage.

Molecular mimicry between *H. pylori* and host

Polypeptides

Although sequence homology between several bacterial polypeptides and those of the host has been demonstrated (Table 1), there is no proof that molecular mimicry plays a causative role in *H. pylori*-associated disease. A lot of work lies between demonstrating sequence homology and showing that molecular mimicry causes damage by eliciting pathogen-induced autoreactive antibodies or T cells.

First, sequence homology must be present. Structure(s) that display mimicry must constitute B- or T-cell epitope, and tolerance must be broken. Second, there should be a correlation between antibody or T-cell levels and disease. Autoreactive antibodies or T cells should be found at higher levels in patients with the disease than in healthy individuals, and it should be possible to detect the antibody, bound to a plausible target, at the site of tissue damage[14]. However, correlation does not prove that the autoreactive T cells or antibodies are actually induced by the microorganism. Alternatively, they might have been elicited in response to host epitopes that have become immunogenic after tissue damage; for example, damage caused by microbial cytotoxins. Finally, correlation does not equal cause-and-effect. Evidence is needed to show that the crossreactive antibodies or T cells play a causative role in host damage and are more than epiphenomena that correlate with the degree of inflammation but do not cause injury. Indications that antibodies or T cells are pathogenic may be obtained by demonstrating deleterious or damaging effects on molecules, cells or organs *in vitro*, either alone or in combination with, for instance, complement. Immunization with purified antigen, passive transfer of specific antibody or adoptive B- or T-cell transfer should elicit the relevant lesions. Infection with bacteria that have the relevant gene(s) knocked out or mutated should cause no lesions, while

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removal of the putative disease-inducing antibodies by plasmapheresis should have a therapeutic effect.

Lipopolysaccharide

LPS of *H. pylori* is unusual in that it expresses Lewis x (Le^x) and y (Le^y) blood group antigens[15–18] (Fig. 2). Such antigens are also expressed on certain host cells and tissues, including gastric mucosa[19]. A survey of 150 strains of *H. pylori*, obtained from three continents, has shown that 85% express Lewis antigens[20].

The epitope specificity of human and animal antibodies to *H. pylori* LPS has been analysed in solid-phase immunoassays with (semi-)synthetic Le^x (CD15) and Le^y antigens[21]. Mice and rabbits immunized systemically with *H. pylori* strains yield high anti-LPS, Le^x and/or Le^y titres. Furthermore, mice infected orally with an *H. pylori* strain expressing Le^x (Ref. 21) develop serum antibodies against Le^x. Of a panel of 30 monoclonal antibodies (mAbs) prepared from mice immunized with *H. pylori*, eight were specific for Lewis antigens (see below)[2,21]. Humans infected with *H. pylori* have been shown to produce antibodies to Le^x and *H. pylori* LPS (Ref. 21), indicating that *H. pylori* LPS acts as an immunogen in humans and other animals and is able to elicit potentially crossreactive antibodies.

H. pylori-induced anti-Lewis antibodies

H. pylori-induced anti-Lewis mAbs have been shown to react strongly with murine and human gastric mucosa by immunohistochemical techniques[21]. The mAbs react with gastric mucus, with foveolar and glandular epithelial cells and also with parietal cell canaliculi, a pattern of reactivity that is similar to that obtained with the sera from *H. pylori*-infected patients[2,3]. By fluorescence-activated cell sorting (FACS), it has been shown that an *H. pylori*-induced anti-Le^x mAb reacts with human polymorphs, which express Le^x (Ref. 21). Gastric mucin, as well as gastric H^+K^+-ATPase, also expresses Lewis antigens[21]. Gastric H^+K^+-ATPase is a well-recognized target of gastric autoimmunity in pre-anaemic corpus-restricted atrophic gastritis[5].

The presence of *H. pylori* crossreactive autoantibodies correlates statistically with the presence and degree of inflammatory cell infiltration in the gastric corpus and with the severity of gastric atrophy[3]. The neck zone of the glands, where the autoantibody reactivity is the strongest, is also the site of the highest influx of

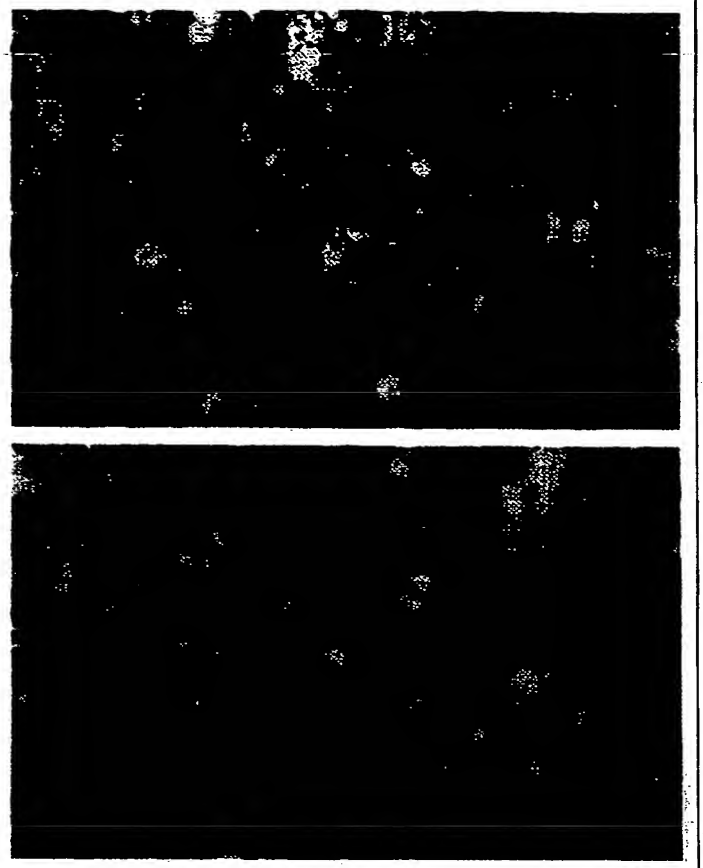

Fig. 1. Binding of *Helicobacter pylori* patient serum with parietal cell canaliculi. **(a)** The glandular area of the gastric corpus is seen as a series of transversely cut tubes, the walls of which are formed by glandular cells (with blue nuclei). The parietal cells (brown) secrete acid into the lumen (marked with a star) of the tubes, which connect to the gastric lumen. The canaliculi are seen as dark brown structures within the parietal cell (marked by an arrow). **(b)** Binding of patient sera after absorption with *H. pylori* cells. Scale bar = 10 μm.

Table 1. Sequence homology between *Helicobacter pylori* and host polypeptides

Bacterial polypeptide	Host polypeptide	Role of mimicry in pathogenesis
hpCopA, hpCopP (ATPases involved in heavy metal ion transport)[7], 686-bp amino acid ATPase[8], vacA (vacuolating toxin)[9], urease β chain[10]	Gastric H^+K^+-ATPase	Not investigated
HspB, 60-kD heat-shock protein[11]	60-kD host heat-shock protein	Unlikely or unproven[a]
Haemagglutinin/protease (hap)[12]	Carbonic anhydrase	Not investigated

[a]The role of *H. pylori* Hsp in inducing pathogenic anti-self antibodies and T cells is not well investigated; therefore, this conclusion is also based on data from other bacterial species. It was concluded[11] that antibodies against Hsp are unlikely to be causatively involved in the pathogenesis of autoimmune diseases. There is no proof that *H. pylori* Hsp-specific T cells induce autoimmune reactions; one study showed that *H. pylori* Hsp-specific T cells did not recognize human Hsp60 (Ref. 13).

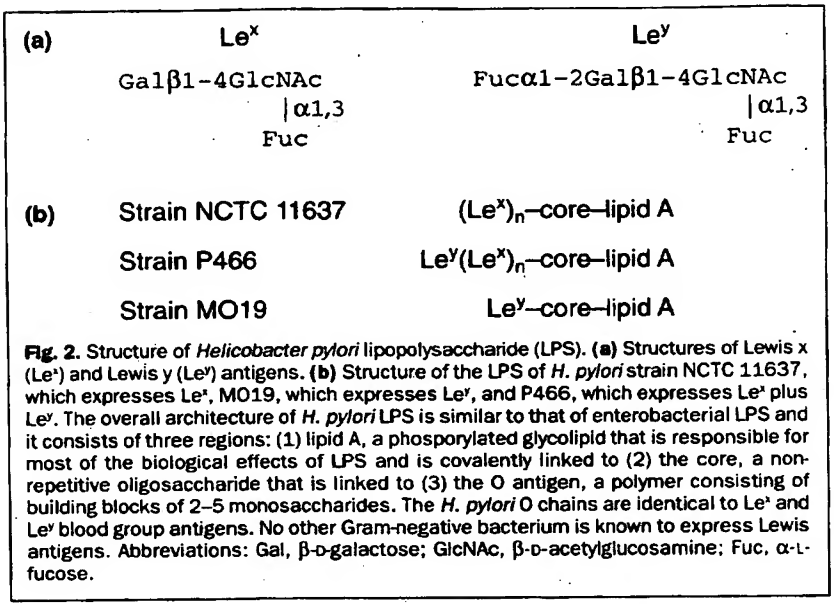

Fig. 2. Structure of *Helicobacter pylori* lipopolysaccharide (LPS). **(a)** Structures of Lewis x (Le^x) and Lewis y (Le^y) antigens. **(b)** Structure of the LPS of *H. pylori* strain NCTC 11637, which expresses Le^x, MO19, which expresses Le^y, and P466, which expresses Le^x plus Le^y. The overall architecture of *H. pylori* LPS is similar to that of enterobacterial LPS and it consists of three regions: (1) lipid A, a phosporylated glycolipid that is responsible for most of the biological effects of LPS and is covalently linked to (2) the core, a non-repetitive oligosaccharide that is linked to (3) the O antigen, a polymer consisting of building blocks of 2–5 monosaccharides. The *H. pylori* O chains are identical to Le^x and Le^y blood group antigens. No other Gram-negative bacterium is known to express Lewis antigens. Abbreviations: Gal, β-D-galactose; GlcNAc, β-D-acetylglucosamine; Fuc, α-L-fucose.

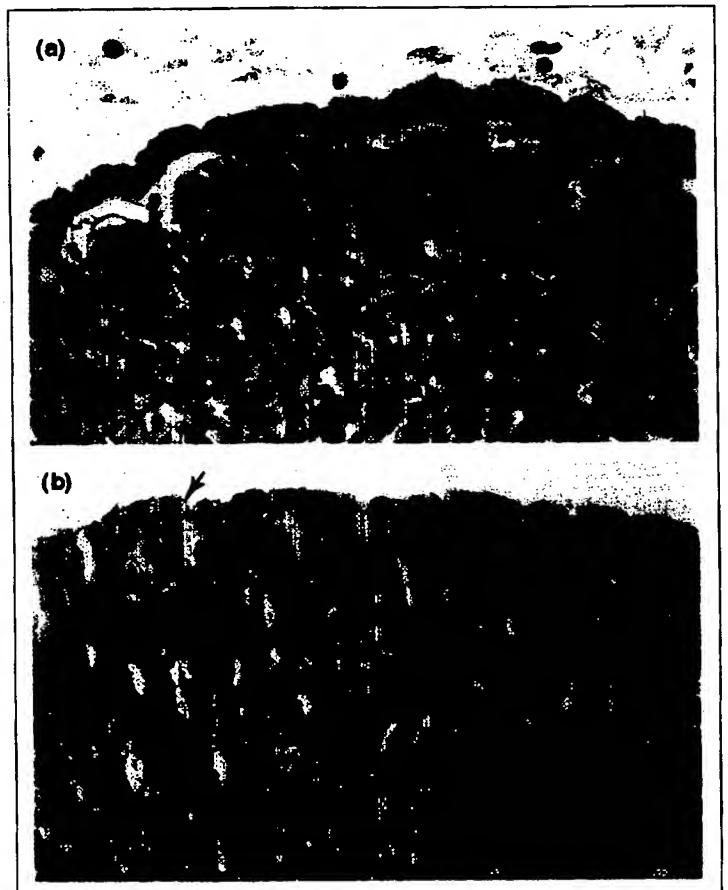

Fig. 3. Histology of the gastric mucosa of mice in which hybridomas were grown that **(a)** secreted monoclonal antibodies (mAbs) against Lewis y antigens or **(b)** secreted a control mAb. The mucosa from mice bearing anti-Lewis y hybridoma contains more lymphocytes (**a**, arrow) and lacks the mucin-producing cells seen in the mucosa of the control mice (**b**, arrow). Scale bar = 20 μm.

inflammatory cells[3]; Le^x and Le^y antigens are strongly expressed in this zone[19]. It is, however, possible that the autoreactive antibodies are not induced by *H. pylori* LPS but by gastric tissue that has become immunogenic upon damage resulting from infection.

Strains isolated from *H. pylori*-infected patients with severe atrophy all express Lewis antigens and induce antibodies that react with gastric mucosa[3]; in contrast, strains from patients with only minimal damage rarely express Lewis antigens and induce less autoreactivity[3]. The observations on patients with severe gastric atrophy suggest, but do not prove, a causative relationship between the expression of Lewis antigens, induction of autoantibodies and mucosal damage; strains isolated from patients with a near-normal mucosa may lack more than the Lewis antigens and may be defective in, for example, cytotoxin-associated gene A (*cagA*) expression[22]. Thus, differences in mucosal damage may not be caused by the expression of Lewis antigens alone.

Concrete evidence that antibodies against Lewis antigens are not merely correlated with inflammation, but can actually induce it, stems from studies in mice[2,21] (Fig. 3). A hybridoma secreting anti-Le^y-specific mAbs and obtained from *H. pylori*-immunized mice was grown in mice that were not infected with *H. pylori*. This procedure leads to high concentrations of circulating mAb. Histological examination of the murine gastric mucosa showed histopathological changes in the surface epithelium, including mucin depletion, increased mitotic activity, an increasing number of immature foveolar epithelial cells and an influx of inflammatory cells. This histological picture is similar to that of *H. pylori*-associated chronic gastritis.

The mechanism by which *H. pylori*-induced anti-Lewis antibodies exert these effects is not known. The eukaryotic parasite *Schistosoma mansoni* of humans also expresses Le^x on its surface[23], and antibodies against Le^x are present in infected patients[24]. These antibodies lyse Le^x-carrying target cells, such as polymorphs, in a complement-dependent pathway[24]; a similar mechanism might operate during *H. pylori*-mediated inflammation. Monoclonal antibodies against Le^x influence the adhesive properties of polymorphs and activate them, which may subsequently lead to tissue damage[25]. In addition, it has been suggested that mAbs against Le^y are taken up by cells and subsequently disturb cell function[26].

Conclusions

Molecular mimicry between *H. pylori* and the host is well established, as is the appearance during infection of antibodies that react with the gastric mucosa. However, how often gastric autoantibodies are a direct consequence of molecular mimicry is unclear. One study[27] found that antibody titres to *H. pylori* correlated with antibody titres to gastric H^+K^+-ATPase, although the response to the latter antigen was not diminished by

prior absorption with *H. pylori* cells. These data suggest that the autoantibodies were not a result of mimicry. Alternatively, the LPS of the cells used for the absorption experiment may not have expressed Lewis antigens. Loss of O antigens after passage of *H. pylori* has been described[28], and strains lacking Lewis antigens do occur[20].

Although the presence of autoantibodies has been shown to correlate with the degree and location of inflammation, whether such a correlation exists between anti Le^x and/or Le^y antibodies and pathology remains to be seen.

Finally, as discussed above, high levels of circulating Le^y mAbs can cause histopathological changes. However, these results need further independent and detailed verification. As this system is artificial, stronger evidence for the pathogenic effects of anti-Lewis antibodies will require more-subtle studies, for instance infection with *H. pylori* knockout strains expressing mutated LPS that does not display mimicry.

Questions for future research

- Are *H. pylori*-induced autoreactive anti-Lewis antigens pathogenic?
- Is it possible to demonstrate the presence of autoantibodies already bound *in vivo* to their targets in human gastric mucosa? Until now, demonstration of the reactivity of autoantibodies has been performed with sections of gastric mucosa incubated with patient sera *in vitro*.
- Is *H. pylori* LPS able to induce a specific T-cell response? T cells to crossreactive epitopes may also cause autoimmune damage. An increasing number of bacterial carbohydrate antigens has been identified that elicits a T-cell response[29].
- Do host genetic factors affect the pathogenic effects of *H. pylori*-induced autoreactivity with regard to the severity of the damage? Major histocompatibility complex background can influence *Helicobacter felis* and *H. pylori*-induced inflammation[30].
- Does epitope spreading take place from Lewis antigens to non-crossreactive protein epitopes? Epitope spreading has been described during autoimmunity[31]. In *H. pylori* infection, it could lead to induction of host-reactive but *H. pylori*-unreactive antibodies.
- Is pernicious anaemia initiated by *H. pylori* infection? Gastric H^+K^+-ATPase is the major target of the autoimmune response in pernicious anaemia[5]; the autoreactivity may start with antibodies against Lewis antigens and spread later to non-crossreactive protein epitopes on the ATPase.

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